sequences in the genomes of turkeys and other birds. Although such observations conform to those made with other, well-documented viral oncogenes, as yet there is little genetic or biochemical evidence for the function of *rel*. A subgenomic mRNA containing *rel* sequences has recently been observed in REV-T-infected cells, implying that the product of *rel* may be unlinked to the products of viral structural genes. In the absence of *rel*-specific antisera, the only available clue to the nature of the *rel*-gene product is the provisional finding of a 58,000-dalton, in vitro translation product of the fragmented genome of REV-T (M. Lai, pers. comm.). For reasons stated earlier (Section II.A.1), it is necessary to exercise caution before ascribing this protein to *rel*.

I. *sis*: Oncogene of Simian Sarcoma Virus

Simian sarcoma virus (SSV), the only transforming retrovirus isolated from primates to date, has been difficult to characterize because of the great excess of helper virus (SSAV) over SSV in conventional stocks. Application of recombinant DNA methods to the study of SSV has recently provided a physical definition of a segment of the viral genome derived from a conserved cellular sequence and apparently unique to SSV. This region of the SSV genome has been called *sis*, but there is no biochemical or genetic definition of its products or functions.

Identification of *sis* has proceeded by the molecular cloning of either unintegrated closed circular DNA (Gelmann et al. 1981) or proviral DNA (Robbins et al. 1981). Using restriction maps in concert with R-loop studies, both groups have judged the ~5.5-kb genome of SSV to be defective for most or all of the replication genes of SSAV, with a substitution of about 1 kb (*v-sis*) positioned close to the 3′ end of viral RNA. Attempts to anneal *v-sis* DNA to a variety of other *onc* sequences have been unsuccessful, sustaining the premise that *sis* is a novel oncogene, unrelated to those described previously (F. Wong-Staal et al., pers. comm.). The position of *sis* within the SSV genome can be construed as a likely indicator that the gene will be expressed independently, not as a component of fused genes; experience with other oncogenes suggests that the product of *v-sis* may therefore be particularly difficult to identify.

III. THE ORIGINS OF VIRAL ONCOGENES

The oncogenes of retroviruses are genetic luxuries. In no known instance is the function of an oncogene required for virus replication, and numerous strains of retroviruses have survived the hazards of evolutionary selection without benefit of an oncogene. How then, and from where, did these genes arise? The quest for answers to these ostensibly abstruse questions has led retrovirologists to what could be the heart of malignant transformation. It now appears that retroviral oncogenes originated from normal genes of vertebrate cells (designated here by the generic term *c-onc*), that oncogenes and their vertebrate progenitors remain closely related if not identical, and that the functions of *c-onc* genes presage the effects of viral oncogenes on infected cells. The discovery of *c-onc* genes has unveiled a family of cellular genes whose alteration or anomalous expression may underlie many forms of oncogenesis (Table 9.2).

A. Emergence of the Thesis: First Clues and Hypotheses

The virus isolated from a chicken sarcoma by Rous did not spring quickly or easily into view. Rather, an infectious tumorigenic agent was obtained from extracts of tumor tissue only after the original sarcoma had been passaged repeatedly from one bird to another (Rous 1911). It seems possible, in retrospect, that the original tumor was not the consequence of virus infection; the sarcoma virus that eventually emerged may not have been present in the tissue with which Rous began his work. The isolation of MSVs (Harvey 1964; Moloney 1966) and Ab-MLV (Abelson and Rabstein 1970a,b) decades later raised these issues in a more explicit manner: The new viruses appeared during the passage of leukemia viruses in rodents, as if new capabilities for pathogenesis could be acquired from the host animal.

The discovery of endogenous retroviruses in chickens (Robinson 1978) and mice (Aaronson and Stephenson 1976) (see Chapter 10), and the development of inbred lines of mice whose predisposition to leukemia appeared to involve genetically transmitted retroviruses (Rowe 1973), added appreciably to these inferences and engendered the "oncogene hypothesis" of Huebner and Todaro (1969; Todaro and Huebner 1972). According to this hypothesis, carcinogens of

Table 9.2 The *c-onc* genes of retroviruses[15]

v-onc[a]	Probable species of origin[b]	*c-onc*	References	*c-onc* expressed[c]	Protein product of *c-onc*[d]
v-src	chicken/quail[e]	*c-src*	Stehelin et al. (1976b)	yes	$pp60^{c-src}$
v-rel	turkey	*c-rel*	Wong and Lai 1981	yes	?
v-myc	chicken	*c-myc*	Roussel et al. (1979); Sheiness and Bishop (1979)	yes	?
v-erb-A	chicken	*c-erb*-A[f]	Roussel et al. (1979); Vennstrom and Bishop 1982	yes	?
v-erb-B	chicken	*c-erb*-B[f]	Roussel et al. (1979); Vennstrom and Bishop 1982	yes	?
v-myb	chicken	*c-myb*	Roussel et al. (1979); Souza et al. (1980)	yes	?
v-fps	chicken	*c-fps*[g]	Shibuya et al. (1980); H. Hanafusa (pers. comm.)	?	?
v-yes	chicken	*c-yes*	Yoshida et al. (1980)	?	?
v-mos	mouse	*c-mos*	Frankel and Fischinger (1977)	n.d.[h]	?[i]
v-abl	mouse	*c-abl*	Goff et al. (1980)	yes	$p150^{c-abl}$
v-bas	mouse	*c-bas*[k]	Andersen et al. (1981)	yes	$p21^{c-bas}$
v-ras	rat	*c-ras*[j]	Ellis et al. (1980)	yes	$p21^{c-ras}$
v-fes	cat	*c-fes*	Frankel et al. (1979)	yes	$p92^{c-fes}$
v-fms	cat	*c-fms*	C. Sherr (pers. comm.)	?	?
v-sis	woolly monkey	*c-sis*	Favera et al.(1981)	?	?

[a]The names of viral genes are treated here as generic terms, although in most instances several separate virus isolates are known (see Table 9.1).

[b]The probable species of origin is inferred from the host in which the particular oncovirus first emerged.

[c]Expression is defined as either detection of transcription from the *c-onc* or detection of a protein encoded by the locus.

[d]Question marks indicate that suitable analyses have not been completed.

[e]Some strains of RSV have been generated experimentally in quail (Wang et al. 1979), but all field isolates of *v-src* have come from chickens.

[f]The separate domains of *erb* (A and B) are represented by similarly separate domains in the chicken genome.

[g]The *c-fps* of chicken is apparently related to *c-fes* of cats (Shibuya et al. 1980).

[h]Fairly extensive efforts have failed to detect transcription of *c-mos* (Frankel and Fischinger 1976).

[i]Failure to detect transcription from *c-mos* raises the possibility that *c-mos* is inactive unless transferred into a retroviral genome.

[j]Rat DNA contains at least two small distinct gene families related to *v-ras*. One family is apparently the source of Harvey and Rasheed *v-ras;* the other family is the source of Kirsten *v-ras* (DeFeo et al. 1981).

[k]The *c-bas* of mice is closely related to *c-ras* of rats (Andersen et al. 1981).

[15]*See Chapter 9 Supplement for revision of this table.*

many types act by inducing the expression of otherwise cryptic retroviral genes already resident in the genomes of the target cells. The oncogene hypothesis is no longer regarded as strictly correct, but it fueled two lines of inquiry. On the one hand, numerous efforts have been made to implicate the induction of retroviruses in the oncogenic mechanisms of chemical and physical carcinogens (Freeman et al. 1973). These experiments have produced enigmatic results, at best. More importantly, however, the oncogene hypothesis was a major heuristic stimulus that prompted experimentalists to ask whether normal cellular DNA might contain retroviral oncogenes. We now know that vertebrate cells do harbor genetic loci homologous to retroviral oncogenes, but these loci are cellular, not viral, genes, and the oncogene hypothesis has been eclipsed by even more sweeping views of the nature of these cellular genes.

B. Discovery of *c-onc* Genes

The search for oncogenes in cellular DNA began with the use of molecular hybridization, following the strategy illustrated by Figure 9.6. The strategy exploited naturally occurring deletions that remove most or all of *v-src* (but no other viral gene) from the genome of RSV and render the virus transformation defective (Duesberg and Vogt 1970; Martin and Duesberg 1972; Lai et al. 1973). Viral RNA bearing this class of deletions could be employed to isolate radioactive DNA ($cDNA_{src}$) that hybridized only with nucleotide sequences encoding (or related to) *src* (Stehelin et al. 1976a). The result was a

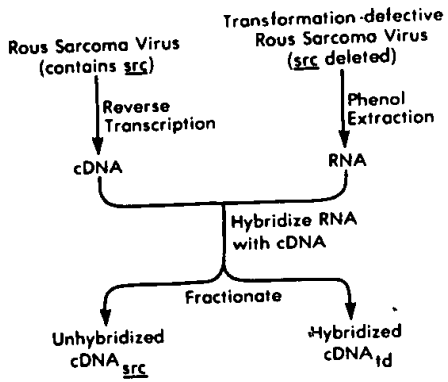

Figure 9.6 Strategy for the preparation of $cDNA_{src}$. The figure outlines the preparation of $cDNA_{src}$. (For details, see Stehelin et al. 1976a).

reagent that provided specificity and sensitivity sufficient to detect a single genetic locus among the immense complexity of vertebrate DNA. Similar cDNAs were prepared for replication-defective MSVs (Scolnick et al. 1973, 1975; Frankel et al. 1976), but the genetic definition of these reagents was less rigorous because suitable deletion mutants were not available for isolation of the cDNAs. As a consequence, the experimental strategies had to rely on the assumption that nucleotide sequences not present in the genome of the helper virus must perforce represent portions of the oncogene, an assumption that proved useful, but not inevitably correct (see below).

The initial findings with $cDNA_{src}$ for RSV prefigured subsequent conclusions for virtually all retroviral oncogenes. Each family of vertebrates examined, including fish, birds, and mammals, displayed evidence of both DNA and RNA related to the *src* gene (Stehelin et al. 1976b; Spector et al. 1978a). The DNA related to *v-src* appeared to occur as only one or very few copies in each haploid portion of vertebrate genomes. Retrovirologists had obtained their first glimpse of the cellular gene we now know as *c-src*.

The mere fact that homologous DNA could be detected across such large phylogenetic distances indicated that the genetic locus or loci in question were highly conserved during the course of evolution. More recent findings have dramatized the extent of this conservation by demonstrating homology with *v-src* (and several other *v-onc*s) in the DNA of *Drosophila* (Shilo and Weinberg 1981b). Conservation of *c-src* was also explored by evaluating the thermal stability of molecular hybrids formed between $cDNA_{src}$ and DNAs from various sources. The results indicated that the nucleotide sequences of *c-src* might diverge by no more than 10–15% from fish to chicken genomes, on the one hand, and from chicken to human genomes on the other hand (Stehelin et al. 1976b; Spector et al. 1978a). The full implications of these findings were not easily sustained at the outset, largely because no assay was available for the protein product of *src*. Nevertheless, it appeared that vertebrate species possessed a highly conserved and expressed (i.e., transcribed) gene that is closely related to a viral oncogene. The strong evolutionary conservation of this gene, and the fact that it was found to be expressed in every tissue and every species examined, signified an essential function in cellular metabolism. These early deductions were later validated and extended by the identification and charac-

terization of a protein encoded by *c-src* (and known as $pp60^{c-src}$; see below).

Difficulties did arise, however, from the use of a less-well-defined cDNA for the oncogene of Ha/Ki-MSV (*v-ras*). Initial results indicated that *v-ras* was related to (and presumably derived from) nucleotide sequences in the genome of an endogenous retroviruslike element of rats (Scolnick et al. 1973; Scolnick and Parks 1974), a troubling deduction, since it stood in striking contrast to the mounting evidence that other retroviral oncogenes are derived from conserved cellular genes. The advent of molecular cloning to the study of retroviral genomes quickly resolved the apparent anomaly. It now appears that the genome of Ha/Ki-MSV was constructed with three distinct components (Ellis et al. 1980) (Chapter 4). One component was derived from the murine helper virus that was used to initiate recovery of the sarcoma virus and was isolated together with the sarcoma virus; a second was derived from an endogenous virus of rats; and a third, the oncogene proper, was derived true to form from a cellular gene of the rat in which the sarcoma virus originally arose.

The principles first enunciated for *src* have since been shown to be widely applicable to retroviral oncogenes (Table 9.2): homologs of these genes (i.e., *c-onc* genes) can be found in vertebrate DNA, many (but apparently not all) of which are expressed in phenotypically normal cells. The sole exception at present is the oncogene of spleen focus-forming virus (SFFV), which appears to be a recombinant form of the retroviral *env* gene, rather than the derivative of a cellular gene (Oliff et al. 1980). All of the identified *c-onc* genes are found in more than one vertebrate species, but the extent of evolutionary conservation varies from one *c-onc* to another: Some are readily detectable only in closely related species, whereas others appear to have taken form in the earliest vertebrates and to have evolved thereafter in concert with speciation. However, these variations may be only matters of degree; it is now reasonable to suppose that every *c-onc* represents a genetic lineage that extends throughout the vertebrate phyla and, in at least some instances, farther down the phylogenetic hierarchy.

The kinship between retroviral oncogenes and cellular genes is certain. But how can we discern parent from progeny? Phylogenetic patterns provide a clue. In contrast to the evolutionary conservation of the cellular genes, the viral oncogenes are usually (although not inevitably; see below) restricted to single strains of retroviruses that were isolated from particular species. Moreover, the homology between the viral oncogene and cellular DNA is greatest for the species in which the oncogene allegedly originated. The most straightforward interpretation of these findings is that retroviral oncogenes are derived from cellular genes. The widespread acceptance of this scheme and the remarkable similarity between retroviral oncogenes and their cellular homologs (described below) have engendered a standard nomenclature described in the introduction to this chapter. The nomenclature is only a convenience, however, and should not be construed as indicating that homologous viral and cellular genes are necessarily identical in either structure or function. The precise relationship between cellular progenitor and viral progeny has yet to be fully explored for any retroviral oncogene.

C. Characterizing *c-onc* Genes

Enumeration of *c-onc* genes by molecular hybridization and by mapping with restriction endonucleases has revealed that many may be unique loci within a given species. However, apparent exceptions exist: (1) the DNA of chickens may contain a second, possibly incomplete locus (a pseudogene) related to *c-src* (Parker et al. 1981); (2) *c-ras* for Ha-MSV is represented by two distinctive loci in rats, one with introns and one without[16] (DeFeo et al. 1981); (3) *v-ras* of the Ki-MSV apparently derives from another cellular gene that is related only distantly to the Harvey *c-ras* (Ellis et al. 1981); and (4) the Rasheed form of *v-ras* apparently derived from a representative of the Harvey *c-ras* family (E. Scolnick, pers. comm.). The last of these findings was unexpected because the relationship of the Rasheed oncogene to the other forms of *v-ras* originally seemed quite distant and was perceived only by serological analyses (Young et al. 1979).

c-onc genes behave as classical Mendelian loci. They occupy constant positions within the genomes of particular species (Hughes et al. 1979a), and they segregate in a predictable fashion when breedings are analyzed with the assistance of structural polymorphisms that have been identified by restriction mapping (D. Spector; B. Vennstrom; both pers. comm.). The loci are recognized by virtue of homology with a viral oncogene, but in most (if not all) instances,

[16] *The same is true for* c-*Ki*-ras.

the homologous nucleotide sequences do not comprise the entire cellular gene. Three major considerations prompt this statement. First, heteroduplex analysis and restriction mapping have demonstrated that the homology between several viral oncogenes and their *c-onc* genes is interrupted by one or more intervening sequences (or introns) in the cellular locus (Goff et al. 1980; DeFeo et al. 1981; Franchini et al. 1981; Parker et al. 1981; Shalloway et al. 1981; Takeya et al. 1981). An example is provided in Figure 9.7, which illustrates a heteroduplex formed by hybridizing DNA representing *v-src* to a portion of *c-src* from chicken DNA; six loops of various sizes are visible, each representing an intron in the cellular locus.[17] The *c-onc* for *v-mos* provides an interesting exception to this rule: The murine and human forms of *c-mos* display uninterrupted homology with *v-mos* in heteroduplex analysis (Jones et al. 1980; Oskarsson et al. 1980; G. Vande Woude, pers. comm.) and by nucleotide sequencing (Van Beveral et al. 1981a). The same is true of at least one of the several rat loci representing *c-ras* (DeFeo et al. 1981). Second, transcription from at least several *c-onc* genes generates RNAs that, even in their mature forms, are appreciably more complex than the homologous viral oncogene (Table 9.2). For example, the mature form of RNA produced from *c-src* is 3.9 kb (Parker et al. 1981), a complexity almost three-fold greater than that of *v-src*. Yet both *v-src* and *c-src* give rise to a protein of 60,000 daltons (Brugge and Erikson 1977; Collett et al. 1978; Oppermann et al. 1979). It appears that large portions of the mRNA for *c-src* may not be translated and that the boundaries of this (or any other) *c-onc* can only be located by applying the definitions that demarcate a transcriptional unit in eukaryotic DNA (i.e., the sites of initiation and polyadenylation). Third, many retroviral oncogenes have been formed by fusing a portion of a replicative gene (typically, *gag*) (see Chapter 4) to nucleotide sequences of a *c-onc*. It seems unlikely that this fusion always incorporates the entirety of the *c-onc* locus into the viral genome. In particular, sequences in the 5′ domain of the *c-onc* may be missing from the viral oncogene.

D. c-onc Genes Are Cellular Genes

The oncogene hypothesis portrayed cellular oncogenes as components of retroviral genomes—a conceptual predisposition that

[17] *The latest count for* c-src *of chicken is 11 introns and 12 exons (see Chapter 9 Supplement).*

proved difficult to override. But we are now certain that *c-onc* genes are cellular genes, not viral genes in disguise. The conclusion rests on three major points of evidence: (1) the location of *c-onc* genes at constant genetic loci in every member of a species (in striking contrast to the diverse distribution and positioning of endogenous retroviral genes) (see, e.g., Hughes et al. 1979a, 1980);

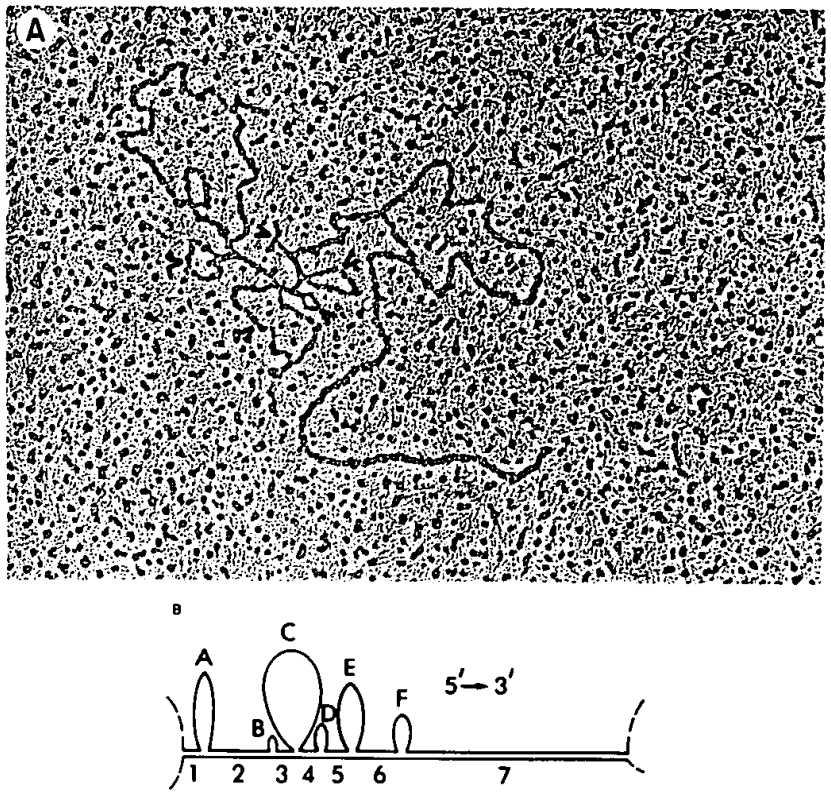

Figure 9.7 Introns in *c-src*. Electron microscopy was used to examine a heteroduplex formed between DNA containing *v-src* and DNA containing *c-src*. The DNAs were prepared by molecular cloning. (*A*) Each loop marked with an arrowhead represents an "intron"—DNA present in *c-src* but not in *v-src* (and presumably not in the mRNA representing *c-src*). (For details, see Parker et al. 1981.) (*B*) Diagramatic representation of the heteroduplex. Dashed lines at each end represent vector DNA. Introns are designated by letters, exons by numbers. The polarity given as 5′—3′ relates the DNA to the plus strand of viral RNA.

(2) the presence of intervening sequences within many of the *c-onc* genes (a hallmark of eukaryotic genes, and, again, in telling contrast to the organization of retroviral genes); and (3) the fact that no *c-onc* has been found within or even linked to a complete or defective provirus of an endogenous retrovirus (Hughes et al. 1979a, 1980; Sheiness et al. 1980b). It seems unlikely that *c-onc* genes were introduced into vertebrate genomes by infection of ancestral species with retroviruses; instead, we must explain how complex cellular genes have made their way partly or entirely into the genomes of preexistent retroviruses.

E. Expression of *c-onc* Genes

The possibility that *c-onc* genes might be expressed in phenotypically normal cells emerged from the discovery of RNA homologous to *v-src* in uninfected fibroblasts of several avian species (Wang et al. 1977; Spector et al. 1978b,c). Similar findings were made subsequently for other *c-onc* genes and other species (Roussel et al. 1979; Sheiness and Bishop 1979; Chen 1980; Bishop et al. 1981). However, some *c-onc* genes may not be expressed; e.g., a thorough-going search has failed to detect transcription from *c-mos* (Frankel and Fischinger 1976; D. Dina, pers. comm.). Efforts to detect transcription from *c-fes* were also unsuccessful (Frankel et al. 1979), but a more recent report described a protein ($pP92^{c\text{-}fes}$) that may be encoded by *c-fes* and that was found in a number of mammalian species (Barbacid et al. 1980a).

Transcription from *c-onc* genes has not been widely studied, but a reasonably coherent set of data is available for four *c-onc* genes representative of avian retroviruses: *c-src*, *c-myc*, *c-erb*, and *c-myb*. Several principles are apparent that may endure even after a greater variety of *c-onc* genes have been studied (Bishop et al. 1981): (1) Each of the *c-onc* genes is transcribed in a variety of tissues and in every species that has been satisfactorily examined (Table 9.3). (2) Transcription from each *c-onc* appears to be independently controlled from one tissue to another (Table 9.4) (Chen 1980; D. Sheiness and B. Vennstrom; D. Stehelin; both pers. comm.); thus, the *c-onc* genes are not coordinately expressed as a group, and the function of each gene may be required only in certain tissues. (3) There is no evidence that transcription from *c-onc* genes is ever coordinated with the expression of endogenous retrovirus genes

Table 9.3 RNAs transcribed from the *c-onc* genes of several avian retroviruses

	RNA size in kilobases						
	c-src	*c-myc*	*c-myb*	*c-erb*			
Chick embryos	3.9	2.5	4.0	12	9.0	4.5	3.0
Chick fibroblasts	3.9	2.5	—	12	9.0	4.5	3.0
Chick bone marrow	3.9	2.5	4.0	—	—	4.5	3.0
Chick yolk sac	3.9	2.5	4.0	—	—	4.5	3.0
Chick brain	3.9	2.5	—				
Chick muscle	3.9	2.5	—				
Chick liver	3.9	2.5	—				
Chick thymus	3.9	2.5	4.0				
Chick bursa	3.9	2.5	4.0				
Chick spleen	3.9	2.5	—				
Duck embryo	3.9	2.5					
Quail embryo	3.9	2.5	4.0				
Mouse fibroblasts	3.9	2.5					
Rat fibroblasts	3.9	2.5					

Data from T. Gonda, D. Sheiness, and B. Vennstrom (all unpubl.). —denotes not detected; blank spaces indicate that data are not available.

(Wang et al. 1977; Spector et al. 1978c). (4) The *c-onc* genes give rise to distinctive RNAs whose sizes have so far proved to be identical in various types of cells and even in different species (Table 9.3). The constancy of these RNAs among widely diverged species testifies to the selective pressures that have apparently preserved the structure and function of *c-onc* genes. As discussed above, most of the transcripts are appreciably larger than would be required to encode the nucleotide sequences that are shared by the homologous viral and cellular genes. (5) Three of the *c-onc* genes (*c-src*, *c-myb*, and *c-myc*) give rise to single mature transcripts (Table 9.3), in accord with the expectation that each locus represents but one gene. Transcription from *c-erb* provides a striking contrast, however, because at least four (and perhaps five) different mature RNAs have been identified, and the distribution of these RNAs varies among different tissues (Table 9.3) (Vennstrom and Bishop 1982). Two of the RNAs are derived from one domain of *c-erb* and the others are from a separate domain; this pattern mirrors the organization of *v-erb* (Sheiness et al. 1981), which is also composed of two independently expressed domains (*v-erb*-A and *v-erb*-B) (see Sheiness et al. 1981; and Chapter 4).

Table 9.4 Transcription of *c-onc* genes in different tissues of the chicken

Tissue or cells	Relative amount of RNA transcribed from *c-onc* genes[a]			
	c-erb	*c-myb*	*c-myc*	*c-src*
Liver	2	<0.2	0.5	0.6
Brain	2	<0.2	0.5	0.7
Embryonic fibroblasts	2	0.2	5	2
Bone marrow	2	30	4	0.4
Yolk sac	4	60	5	<0.3
Macrophages	0.7	<0.2	1	6
Thymus	1	90	5	2
[illegible]ated thymic lymphocytes	0.3	30	15	0.4
Bursa	1	5	2	1
Isolated bursal lymphocytes	0.3	20	10	n.t.
Spleen	1	2	5	6
Isolated splenic lymphocytes	0.3	20	15	n.t.

Data from T. Gonda and D. Sheiness (both unpubl.).
[a]n.t. denotes not tested.

F. Identifying the Proteins Encoded by *c-onc* Genes

RNA transcribed from several *c-onc* genes has been found in polyribosomes and is therefore presumably translated into proteins (Spector et al. 1978b; B. Vennstrom, pers. comm.). The search for these proteins has not been easy. Most cells contain only small amounts of *c-onc* mRNAs and proteins, and the identification of [illegible]*c* proteins has so far depended on the development of antisera that react with the product(s) of the corresponding viral oncogenes, an undertaking that is itself unpredictable and technically demanding.

Four proteins encoded by *c-onc* genes have been identified to date: $pp60^{c-src}$, a 60,000-dalton phosphoprotein specified by *c-src* (Collett et al. 1978, 1979b; Oppermann et al. 1979; Rohrschneider et al. 1979); $p21^{c-ras}$, a 21,000-dalton phosphoprotein encoded by the Harvey *c-ras* (Langbeheim et al. 1980); $p150^{c-abl}$, a 150,000-dalton protein apparently derived from *c-abl* and formerly called NCP150 (Witte et al. 1979b), and $p92^{c-fes}$, a 92,000-dalton protein presumed to be encoded by *c-fes* and formerly called NCP92 (Barbacid et al. 1980a). Both $pp60^{c-src}$ and $p21^{c-ras}$ have been extensively studied,

both are remarkably similar to their viral counterparts in structure and apparent function (Collett et al. 1978, 1979b; Karess et al. 1979; Oppermann et al. 1979; Rohrschneider et al. 1979; Langbeheim et al. 1980; Sefton et al. 1980b; Karess and Hanafusa 1981); and both are found in a large variety of cells and are distributed across wide phylogenetic distances (Oppermann et al. 1979; Rohrschneider et al. 1979; Langbeheim et al. 1980). Much less is known of $p150^{c-abl}$ (Witte et al. 1979b). Its size and composition are different from those of any of the proteins encoded by the several variants of *v-abl*, it has been found in appreciable (but very small) amounts only in thymocytes and other lymphoid cells, its phylogenetic distribution has not been reported, and nothing is known of its function. Surprisingly, RNA transcribed from *c-abl* has been found widely distributed among tissues and cells of many sorts, most of which contain no detectable $p150^{c-abl}$ (D. Baltimore, pers. comm.). This discrepancy cannot presently be explained.

The apparent product of *c-fes* ($p92^{c-fes}$) has been found in the cells of rats and related mammals, but not in rodents or primates (Barbacid et al. 1980a). Little else is known of the protein: the extent of its relationship to products of *v-fes* has not been critically assessed; no function has been identified, and there is no explanation for the failure to find RNA transcribed from *c-fes* in species known to produce $p92^{c-fes}$.

G. How Similar Are Viral Oncogenes and *c-onc* Genes?

Assessment of the similarities between *c-onc* genes and their viral derivatives has taken two general forms: comparison of the nucleotide sequences that embody the genes and comparison of the proteins encoded by the genes. Early studies with molecular hybridization raised the possibility of a substantial kinship between the *c-onc* and viral oncogene, but satisfactory tests of the issue awaited the isolation of the genes by molecular cloning, on the one hand, and identification and characterization of the proteins encoded by the genes, on the other. With either or both of these chores now accomplished in several instances, the evidence mounts for remarkable similarity, if not identity, between the viral and cellular forms of oncogenes:

1. Coding sequences shared by viral and cellular oncogenes have so far been indistinguishable by heteroduplex analysis (Jones et al. 1980; Oskarsson et al. 1980; DeFeo et al. 1981; Parker et al. 1981; Shalloway et al. 1981; Takeya et al. 1981), although ambiguities arise whenever the cellular locus is punctuated by introns.
2. DNA sequencing has permitted an extensive comparison of *v-mos* and *c-mos* (Van Beveren et al. 1981a,b). The first few codons of *v-mos* in Mo-MSV are vestiges of the *env* gene into which *c-mos* was inserted. Otherwise, only occasional nucleotide substitutions distinguish *v-mos* from *c-mos*.
3. The *c-erb* locus is extraordinarily complex, extending over at least 40 kb of chicken-cell DNA and containing a minimum of 12 introns (Vennstrom and Bishop 1982). Nevertheless, when the exons of the locus were mapped against *v-erb*, close homology was observed. The viral gene encodes two proteins in separate domains (Anderson et al. 1980; Sheiness et al. 1981) and the cellular locus displays the same structural organization (Vennstrom and Bishop 1982).
4. The viral and cellular forms of $pp60^{src}$ are remarkably similar (Collett et al. 1978, 1979b; Karess et al. 1979; Oppermann et al. 1979; Rohrschneider et al. 1979; Sefton et al. 1980b; Karess and Hanafusa 1981). They share size, display antigenic cross-reactivities, and yield closely related peptide maps. Both are principally affiliated with the plasma membrane of the cell (Courtneidge et al. 1980). Both are phosphorylated and have similarly disposed phosphoamino acids, with phosphoserine in the proximity of the amino terminus (Collett et al. 1979a,b) and phosphotyrosine within a carboxyterminal domain (Hunter and Sefton 1980; Karess and Hanafusa 1981). Both are protein kinases that phosphorylate tyrosine in substrate proteins (Collett et al. 1979b, 1980; Oppermann et al. 1979; Rohrschneider et al. 1979; Hunter and Sefton 1980; Levinson et al. 1980). The two proteins can be distinguished only by very subtle criteria: Some antisera react with the viral protein, but not the cellular protein, presumably reflecting the fact that the antisera were raised against the viral protein, rather than the cellular protein (Oppermann et al. 1979); the peptide maps of the two proteins differ in a few respects (Sefton et al. 1980b; Karess and Hanafusa 1981); and the phosphotyrosine may be contained within different tryptic peptides in the two proteins (Karess and Hanafusa 1981; Smart et al. 1981) It also remains possible that the viral and cellular proteins respond differently to controlling influences in the cell and that the kinase activities of the two proteins have different substrate specificities. Definitive tests of these important potential distinctions are not presently available.
5. The possibility that *v-src* and *c-src* are functionally similar has received dramatic support from the claim that recombination between *c-src* (in chickens) and deletion mutants of *v-src* can apparently reconstitute a functional oncogene (Hanafusa et al 1977; Wang et al. 1978, 1979; Karess et al. 1979; Vigne et al. 1980; Karess and Hanafusa 1981). In the most telling examples, the allegedly reconstituted oncogene retains a 3′-terminal portion of *v-src* (~25% of the gene) but is otherwise apparently constructed entirely of nucleotide sequences derived from *c-src* (Wang et al. 1979; Karess and Hanafusa 1981). The protein encoded by the reconstituted *v-src* is so similar to both $pp60^{v-src}$ and $pp60^{c-src}$ that its genetic origins are difficult to discern, although peptide maps suggest that $pp60^{v-src}$ of the recombinant virus is indeed a hybrid of both cellular and viral origins (Vigne et al. 1980; Karess and Hanafusa 1981). Even these findings cannot assure us that $pp60^{c-src}$ and $pp60^{v-src}$ are functionally equivalent, however; in every instance, the recombinant viral protein has derived at least 20% of its carboxyterminal domain from the parental virus in the recombination (Wang et al. 1979; Karess and Hanafusa 1981), and it is the carboxyterminal domain of $pp60^{v-src}$ that bears the protein kinase activity (Levinson et al. 1981; Oppermann et al. 1981b). Moreover, an extensive analysis of oligonucleotides from *src* in different strains of RSV failed to find any evidence that could trace the origins of the reconstituted viruses to *c-src* (Lee et al. 1981). It therefore remains conceivable that the reconstituted RSVs arose from recombination among defective viruses or from other forms of RSV contaminating the stocks of deletion mutants used to initiate the experiments.
6. The proteins encoded by Harvey *v-ras* and one of the two closely related *c-ras* genes have been compared in considerable detail and appear to be quite similar. They each have a molecular weight of approximately 21,000, they react with the same antisera, they yield related peptide maps, and they display the same

biochemical function, i.e., the capacity to bind guanine nucleotides with high affinity (Scolnick et al. 1979; Shih et al. 1980). However, they may differ in one regard; pp21$^{v\text{-}ras}$ is phosphorylated on threonine residues, whereas phosphorylation of p21$^{c\text{-}ras}$ has yet to be detected (T.Y. Shih et al. 1979a; Langbeheim et al. 1980; Shih et al. 1980).

7. Functional similarities between *v-onc* and *c-onc* have been demonstrated most persuasively by work with *c-mos* and *c-ras*. Both of these *c-onc* genes have been isolated by molecular cloning (*c-mos* from mouse DNA, *c-ras* from rat DNA) and coupled to a retrovirus long terminal repeat (LTR) in order to favor vigorous expression. Some of the cells that receive these hybrid genetic units by transfection become transformed to a neoplastic phenotype (Oskarsson et al. 1980; DeFeo et al. 1981), as if the *c-onc*s might carry out the same functions as their homologous *v-onc*s (see also Section K.3).

None of the preceding examples provide a definitive demonstration of identity between the viral oncogene and *c-onc*. But the weight of the evidence now suggests that retroviral oncogenes encode functions also found in normal vertebrate cells. If correct, this conclusion may have significance that reaches far beyond the confines of tumor virology (see below).[18]

The Family of *c-onc* Genes

We presently know of at least a dozen retroviral oncogenes, each distinguished by its nucleotide sequence and each with a corresponding oncogene (see Table 9.1). Moreover, ostensibly similar oncogenes may be the products of related but separate cellular loci. For example, the apparently homologous oncogenes of Ha-MSV and Ki-MSV (*v-ras*), formerly believed to have originated from the same *c-onc*, are now known to be the progeny of two different (albeit related) cellular genes (DeFeo et al. 1981). The total number of *c-onc* genes is therefore likely to grow as efforts to identify novel isolates of retroviruses continue. On the other hand, the number of these genes may not be inordinately large: the *c-onc* genes for *src*, *myc*, *erb*, *myb*, *yes*, and *fps* are each represented at least twice

[18] *There is now extensive evidence that most if not all* c-onc *genes differ from the corresponding* v-onc *in subtle or large ways (see Chapter 9 Supplement).*

among the handful of independently isolated avian retroviruses, *c-fes* is a feline counterpart of *c-fps* that appears in two strains of feline sarcoma virus (Shibuya et al. 1980) and *c-bas* is a murine counterpart of *c-ras* that appears in sarcoma viruses isolated from rats (Andersen et al. 1981). The reiterative emergence of *c-onc* genes in different virus isolates and from different species suggests that we may have the majority of these genes already in view.

Whatever their number, *c-onc* genes might comprise a family of genes whose interrelationships are akin to those found in the multigene families that encode immunoglobulins, histocompatibility antigens, etc. This suggestion stems from the fact that all *c-onc* genes, however diverse in structure, give rise to viral genes with the dramatic property of oncogenicity in common. In fact, there are reasons to believe that the apparent structural diversity of *c-onc* genes may obscure common origins and related functions: (1) The nucleotide sequences of *v-src* and *v-mos* are very different, yet the amino acid sequence encoded by these genes reveals significant homologies that indicate a common ancestor (Van Beveren et al. 1981a); (2) several different *v-onc* genes (and so far as we know, their *c-onc* genes as well) encode tyrosine protein kinases (Feldman et al. 1980; Reynolds et al. 1980; Witte et al. 1980a; Neil et al. 1981c), and these enzymes may affect similar sets of cellular proteins (Cooper and Hunter 1981a,b; T. Hunter, pers. comm.).

Given the apparent functional relationships among the identified *c-onc* genes, it is of interest to know whether these genes might be clustered or linked in the cellular genome. The available data are in conflict. Fractionation of chicken chromosomes by rate-zonal centrifugation has located *c-src* on one of the smaller macrochromosomes (Padgett et al. 1977; Hughes et al. 1979b), *c-myc* on one of the two or three largest chromosomes (Sheiness et al. 1980b), and *c-erb* on a chromosome of intermediate size[19] (B. Vennstrom, pers. comm.). In contrast, hybridization in situ indicated that *c-src*, *c-myc*, *c-myb*, and *c-erb* are all located on one or another of the chicken microchromosomes (Tereba et al. 1979; A. Tereba, pers. comm.). The discrepancies may arise from the fact that the cells used for chromosome fractionation are neoplastic and contain at least one chromosomal translocation. On the other hand, it may not be necessary that all *c-onc* genes be genetically linked. For example, demonstrably related genes (such as α- and β-globin genes and genes whose func-

[19] c-erb-A *is located on a minichromosome in chickens, and* c-erb-B *is on a large chromosome (see Chapter 9 Supplement).*

tions are coordinately induced by estrogen) are located on different chromosomes in the chicken (Hughes et al. 1979b).

I. Mechanisms of Genetic Mimicry: Genesis of Retroviral Oncogenes

By what means have *c-onc* genes been acquired by viral genomes? Two competing answers to this question have emerged:

1. It is possible that each retrovirus arises fully grown from the rearrangement and permutation of cellular genes (Temin 1980). This account is a restatement of Temin's original "protovirus hypothesis" (Temin 1974) and suggests that oncogenes may be present from the inception of certain retroviral genomes.
2. It is more generally assumed that preexistent retroviruses assimilate *c-onc* genes by recombination (Bishop 1981). Several lines of evidence conform to (but do not prove) this explanation. First, large (but never complete) deletions in *v-src* can be repaired by recombination with *c-src* in chickens (see above). It is by no means certain, however, that the mechanism of this recombination provides a general explanation for the transduction of *c-onc* genes. Second, the oncogenes of several murine retroviruses (*v-ras, v-mos,* and *v-abl*) appeared during the passage of leukemia viruses in rodent hosts (Harvey 1964; Moloney 1966; Abelson and Rabstein 1970a,b). We presume, but cannot prove, that here the experimentalist may have reproduced the events that give rise to *v-onc* genes in the wild. Third, several investigators have reported deliberate and apparently successful efforts to transduce *c-onc* genes by infection of cells in tissue culture with retroviruses that do not initially contain oncogenes (Rapp and Todaro 1978, 1980; Rasheed et al. 1978; Stavnezer et al. 1981). These efforts have produced retroviruses with varied and novel oncogenic potentials, but the genetic bases of most of these potentials have yet to be elucidated.

Neither of the preceding views offers a persuasive account of the mechanism by which the transduction of *c-onc* genes actually occurs. The protovirus hypothesis now relies mainly on the possibility that retroviruses are produced by the antics of ancestral transposable elements (Temin 1980). The evidence for this possibility is limited and circumstantial. But we can do little better at suggesting how *c-onc* genes might be recombined into preexistent retroviral genomes. In particular, homologous regions that could facilitate the putative cross have not been identified, and we need to explain how the introns of *c-onc* genes are removed to generate the uninterrupted coding units of *v-onc* genes. Recent work by Goldfarb and Weinberg (1981b) may have provided a pertinent experimental model, however, by demonstrating that retroviruses may participate in illegitimate recombination, so long as RNAs representing the genetic elements to be recombined are first encapsidated and rendered infectious for susceptible host cells.

Other puzzles remain, as well. Is the seizure of *c-onc* genes a unique event, or might retroviruses be generalized transducing agents whose acquisition of more prosaic genes is merely less likely to be perceived? Are there selective pressures that favor the transduction of *c-onc* genes and their retention by retroviral genomes? When oncogenes are formed by fusing *c-onc* to a portion of a viral structural gene (as is frequently the case; see Chapter 4), what portion of the cellular locus actually joins the viral genome and what influence does the hybrid nature of the resulting oncogene essential for oncogenicity? (The hybrid genetic structure appears not to be necessary for tumorigenesis: at least two oncogenes—*v-myc* and *v-myb*—occur as both hybrid genes and as independently expressed loci not fused with *gag*.) And how are we to interpret the unusual nature of the oncogene for SFFV? Is it an exception to an otherwise pervasive rule or does it signify that the origins of retroviral oncogenes are more diverse and more complex than we presently realize?[20]

J. Are *c-onc* Genes Useful to Normal Cells?

It has become an article of faith that *c-onc* genes serve essential purposes in uninfected cells. Why else would these genes have been conserved over long periods of evolutionary time and why else would many of their numbers be expressed in both embryonic and adult tissues? The inference is easy to draw but difficult to explore, and the difficulty lies less with biochemical function than with cellular physiology. Once $pp60^{v\text{-}src}$ was known to be a protein kinase, the

[20] *Further analysis of tumorigenesis by SFFV has made the contrast less troubling (see Chapter 9 Supplement).*

demonstration of a similar enzymic activity associated with $pp60^{c-src}$ followed in short order (Collett et al. 1979b; Oppermann et al. 1979; Rohrschneider et al. 1979). But how does this enzymic activity, or for that matter, the biochemical function of any other *c-onc*, serve the metabolism of the normal cell? The question is usually answered by reasoning that the actions of *v-onc* genes mirror the functions of *c-onc* genes. The cell transformed by a retroviral oncogene divides incessantly. Might the homologous proto-oncogene therefore be a normal effector of cell division? Many (perhaps all) retroviral oncogenes arrest, reverse, or otherwise disturb the normal course of cellular differentiation (Graf and Beug 1978; Boettiger and Durban 1980; Baltzman and Levine 1981). Might their counterparts in normal cells be regulators of growth and development and, if so, might the lineages in which they are normally active dictate the kinds of cells that are vulnerable to transformation by the homologous viral oncogenes? Experimental data that relate to these issues are sparse and enigmatic:

1. Since *v-src* transforms fibroblasts, it is conceivable that expression of the cellular homolog *c-src* might vary in concert with changes in cell growth. To date, efforts to sustain this expectation have failed. For example, the expression of *c-src* remained unchanged throughout the course of experiments in which the growth of fibroblasts was first arrested for as long as 2 weeks by serum deprivation and then stimulated by restoration of serum to the growth medium (Spector et al. 1978a).

2. Efforts to discern preferential expression of *c-onc* genes in specific tissues have so far failed to yield coherent results. Some loci (such as *c-src*, *c-myc*, and *c-erb*) are active at low or intermediate levels across a broad spectrum of tissues, whereas the activities of others (e.g., *c-myb*) are more restricted in their distribution (Table 9.4). In most instances, the distribution of activity does not conform to predictions based on the pathogenicities of the corresponding oncogenes. The most provocative finding at present comes from Scolnick et al. (1981): Primitive hematopoietic cells (pluripotent CFU-S cells), but not cells of other origins, contain large amounts of $p21^{c-ras}$, a protein whose viral homolog is apparently capable of inducing erythroleukemias as well as sarcomas.

K. Paradox of Neoplastic Transformation by Retroviral Oncogenes

If retroviral oncogenes embody functions found also in normal vertebrate cells, why do the oncogenes induce abnormal phenotypes in infected cells? Two possible answers come to mind. First, transformation by retroviruses may be a consequence of dosage. The virus may overload cells with otherwise normal gene products; sustained and abundant expression of the genes, rather than anomalous properties of their products, may lie at the root of tumorigenesis by *v-onc* genes. Alternatively, *v-onc* genes and *c-onc* genes may differ in subtle but important ways. For example, the protein kinase activity of $pp60^{v-src}$ might have unique substrate specificities that could account for neoplastic transformation by RSV.

Although the extent of resemblance between *c-onc* genes and *v-onc* genes has yet to be fully measured, several points of evidence suggest that retroviral oncogenes can transform cells by means of dosage:

1. The dosage of *v-onc* products is indeed large, when compared with the amounts of *c-onc* products found in most cells; fibroblasts transformed by RSV contain about 100-fold more $pp60^{v-src}$ than $pp60^{c-src}$ (Collett et al. 1978; Oppermann et al. 1979), and similar differences have been found for other oncogene and *c-onc* products (Witte et al. 1979b; Langbeheim et al. 1980). The vigor of oncogene expression may be attributable in large measure to the efficacy of the retrovirus promoter for transcription (see Chapter 5).[21]
2. The relatively large dosages of oncogene products are essential to maintain the neoplastic phenotype. On occasion, presently unidentified events in the infected cell can attenuate the synthesis of retroviral RNA and, hence, the expression of viral genes (see Chapter 5). The amounts of oncogene product fall by 10- to 100-fold, and the cell reverts to an ostensibly normal phenotype (Macpherson 1965; Boettiger 1974; Deng et al. 1977; Porzig et al. 1979; Bishop et al. 1980).
3. Molecular clones of two *c-onc* genes (*c-mos* and *c-ras*) have been linked to portions of the MLV genome that encourage viral gene expression (in particular, the LTR). The chimeric DNAs, bearing no portion of a viral oncogene, can transform fibroblasts to a

[21] *See footnote to p. 1082.*

neoplastic phenotype (Oskarsson et al. 1980; Blair et al. 1981; DeFeo et al. 1981; G. Vande Woude; E. Scolnick; both pers. comm.). Cells transformed in this manner by *c-ras* contain relatively large quantities of the gene product, $p21^{c\text{-}ras}$ (DeFeo et al. 1981; E. Scolnick, pers. comm.), thus sustaining the view that amplification of *c-ras* expression suffices to induce the neoplastic phenotype.

L. Does the Homology between *v-onc* Genes and *c-onc* Genes Dictate the Host Range of Viral Transformation?

Neoplastic transformation by retroviral oncogenes is remarkably specific. Pathogenicity for specific tissues is a distinctive property of each strain of retrovirus. In cell culture, oncogenes display a similarly predictable and generally limited range of susceptible cells that correlates well with the actions of the oncogene in infected animals (Graf and Beug 1978). The origins of target-cell specificity, as this phenomenon is known, are uncertain. It is first of all possible that transformation is merely a reflection of susceptibility to infection by different strains of virus. Inference speaks against this possibility. Viral genes that determine the host range of infectivity (such as *gag* and *env*) are shared among families of retroviruses whose spectra of oncogenicities are very different. Moreover, experimental data indicate that cells can be infected by and produce retroviruses bearing oncogenes without necessarily being transformed (Graf et al. 1980; Durban and Boettiger 1981a). These findings have led to the more subtle suggestion that only certain cells are vulnerable to the effects of each oncogene.

What factors could determine cellular vulnerability to transformation by *v-onc* genes? We do not know, but it has been suggested that the kinship of *v-onc* genes and *c-onc* genes might be responsible. The deleterious effects of oncogene dosage might be restricted to cells in which the homologous *c-onc* is normally expressed and effective; alternatively, cells in which the *c-onc* is not usually active might be more vulnerable to the actions of the oncogene. The available (and admittedly provisional) data do not sustain these views. For example, the pattern of *c-onc* expression illustrated in Table 9.4 in no way reflects the spectrum of susceptibility to the homologous viral oncogenes.

M. Do *c-onc* Genes Provide a Common Pathway for Oncogenesis?

The action of viral oncogenes may provide useful analogs for the enzymic mechanisms that give rise to and sustain the malignant phenotype. But it appears that viruses bearing oncogenes are not usually responsible for tumorigenesis in human beings (Pimentel 1979). We should therefore look to the cell itself if we are ever to discern common origins of malignancy. In particular, we need to identify the events that spark the onset of oncogenesis, and we must determine whether a particular cellular gene (or set of genes) always mediates progression to and maintenance of the malignant phenotype.

We do not know how oncogenesis initiates. The matter has elicited great controversy, with some investigators arguing for mutations (Ames 1979; Epstein and Swartz 1981) and others for chromosomal rearrangements, transpositions of DNA, or even reversible epigenetic events (Rubin 1980; Cairns 1981). In contrast, the discovery of *c-onc* genes may have brought to view genes whose actions can mediate oncogenesis, once the cell has sustained an initiating lesion. We have diverse reasons to suspect the existence of such "cancer genes":

1. A number of malignancies have appeared as heritable traits in human pedigrees (Knudson 1981), and it has even been suggested that each of the roughly 100 types of malignancies will eventually be attributable to abnormalities affecting a specific genetic locus (Knudson 1979, 1981).
2. Several efforts have been made to enumerate the genetic loci that might mediate neoplastic transformation by chemical carcinogens. In most instances, the results implicate no more than a few dozen or a few hundred genes as potential mediators of chemical carcinogenesis (Parodi and Brambilla 1977).
3. DNAs extracted from some lines of chemically transformed cells and from certain tumors induce neoplastic transformation when transferred into cells in culture (C. Shih et al. 1979; Krontiris and Cooper 1981; Shilo and Weinberg 1981a). Caveats are necessary The efficiency of transformation is generally quite low; transformation occurs reliably in only a few (and empirically recognized) line of recipient cells (e.g., mouse NIH-3T3); and only a limited number of transformed cell lines or tumors have so far yielded DNA capable of inducing neoplastic transformation. But the

data do suggest that DNAs from at least some forms of neoplastic cells contain stable and heritable changes that are responsible for the malignant phenotype. Moreover, provisional studies with restriction endonucleases indicate that the same domain of DNA may be affected in independent tumors of common type and/or common cause (Shilo and Weinberg 1981a).[22]

4. If first sheared to molecular weights of approximately 0.3×10^6 to 3×10^6, even DNA from normal cells can transform NIH-3T3 cells at a very low frequency, and the transformed mouse cells, in turn, yield DNA that can induce transformation at much higher efficiencies (Cooper et al. 1980), as if the original shearing of normal DNA unleashed a potentially oncogenic gene whose action is now stably established in the transformed mouse cells. Activation of the gene has been attributed to disruption of linkage between the oncogenic gene and a *cis*-active regulator (Cooper et al. 1980).

Are *c-onc* genes the cancer genes of normal cells? Is it the induction of their activities that lies at the root of all forms of oncogenesis? Answers to these questions may come eventually from exhaustive surveys of *c-onc* expression in naturally occurring tumors. At present, we have a single but immensely provocative clue, derived from an unexpected source: the study of tumorigenesis by avian leukosis viruses (ALVs).

As explained in detail in Chapter 8, most of the ALV-induced bursal tumors examined to date contained viral DNA integrated in the vicinity of *c-myc*, and as a seeming consequence of the insertions, the expression of the *c-onc* appeared to have been greatly augmented. Detailed analysis of the bursal tumors has produced three conclusions that are important in the present context: Oncogenesis by retroviruses may not always require viral gene products; the frequency with which ALV proviruses are found in the vicinity of *c-myc* implies that the site of insertion figures in the oncogenic mechanism; and it is likely (but by no means proven) that the heightened expression of *c-myc* provokes and/or sustains the chain of events that eventuates in lymphoid leukosis or renal carcinoma. However, the following questions remain:

1. In a few ALV-induced tumors, viral DNA is not inserted near *c-myc* and expression of *c-myc* has not been induced (Hayward et al. 1981; W. Hayward, pers. comm.). Nevertheless, single (or very few) sites have been used for integration of the ALV provirus in each of these tumors, and it is therefore possible that the insertion of viral DNA has induced the expression of nearby cellular DNA representing an as yet unidentified *c-onc*.
2. The viral oncogene (*v-myc*) derived from *c-myc* has never been reported to cause lymphomas (Moscovici et al. 1978). The implication of *c-myc* in the genesis of B-cell tumors therefore came as a surprise that remains unexplained. However, the induction of lymphomas by ALV follows a protracted course of events that may not spring immediately from the effects of a single gene; the roles of *c-myc* and *v-myc* in oncogenesis may differ greatly. There is evidence to sustain this view (Cooper and Neiman 1980). DNA from lymphoid tumors induced by ALV elicits the neoplastic phenotype when introduced into mouse fibroblasts by transfection. Contrary to expectations, however, the transformed mouse cells contain neither ALV DNA nor *c-myc* derived from the lymphoid tumors (G. Cooper, pers. comm.). Transformation of the fibroblasts must therefore be due to another oncogene, activated in the infected B cells (perhaps by the effects of *c-myc*), and more effective than *c-myc* itself in the transformation of fibroblasts. In this formulation, *c-myc* can be viewed as the initiator of tumorigenesis, the oncogene responsible for transformation of fibroblasts as a potential maintenance function.
3. Is the alleged tumorigenic effect of *c-myc* limited to cells in the B-lymphocyte lineage? Possibly not; for example, provisional data also implicate *c-myc* in the genesis of renal tumors induced by ALV (Cooper and Neiman 1981).[23] It is perhaps significant that these tumors are analogous to the renal carcinomas commonly induced by the action of *v-myc* (Moscovici et al. 1978).
4. Is *c-myc* an inevitable participant in the genesis of B-cell (and perhaps renal) tumors, whatever their initiating cause? The induction of *c-myc* expression has been implicated in lymphomagenesis by another retrovirus that lacks an oncogene, the chicken syncytial virus (CSV) (H.-J. Kung, pers. comm.). Although the CSV genome is not homologous to that of ALV, the similarities of the two viruses are too great to provide a compelling test of the larger issue. The question may be answered properly, however, by forthcoming surveys of *c-myc* expression (as well as expression of other *c-onc* genes) in tumors of various origins.

Do the findings with ALV reveal how other viruses devoid of

[22] *These conclusions were in great part erroneous, since mutant versions of* c-ras *have been found in tumors and leukemias of many types (see Chapter 9 Supplement).*

[23] *The tumor in question was probably a metastasis of bursal lymphoma to the kidney. More extensive efforts to implicate* c-myc *in the genesis of renal carcinoma by ALV have*

oncogenes might cause tumors? The induction of *c-onc* expression by the integration of ALV DNA is a form of insertional mutagenesis, and other integrative viruses are in principle capable of the same. For example, provisional evidence indicates that the integration of RAV-1 DNA in the vicinity of one or both *c-erb* loci may be involved in the genesis of avian erythroblastosis induced by this virus (H.-J. Kung, pers. comm.); and a number of mouse mammary carcinomas induced by mouse mammary tumor virus (MMTV) carry viral DNA integrated in the same domain of cellular DNA (R. Nusse, pers. comm.). Other potential examples abound: the nephroblastoma induced by some strains of MAV-2 (Watts and Smith 1980); thymic leukemia induced by murine leukemia virus (MLV); the leukemias induced by feline, bovine, and ape leukemia viruses; and further afield, leukemogenesis by SV40 virus, the hepatitis-B virus that has been implicated in the genesis of hepatic carcinoma, and even oncogenesis by herpesviruses. If any of these sundry viruses acts by means of insertional mutagenesis, the sites of integration in the DNA of the tumor cells may finger cancer genes not yet identified by other means. Virologists are in hot pursuit of these possibilities, hoping to expand the catalog of cancer genes and to gain insight into the apparent tissue specificity of their actions.

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